Scientific Bulletin. Series F. Biotechnologies, Vol. XVII, 2013
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

EVALUATION OF FRUCTAN CONTENTS IN THE TAPROOTS OF PLANTS *LACTUCA SERRIOLA* L. AND *SONCHUS OLERACEUS* L.

Nadezhda PETKOVA, Panteley DENEV

University of Food Technologies, Department of Organic Chemistry,
26 Maritza Blvd., 4205, Plovdiv, Bulgaria

Corresponding author email: petkovanadejda@abv.bg

Abstract

The current research aimed to present the evaluation of the underground parts of two widespread plants in Bulgaria- prickly lettuce (Lactuca serriola L.) and annual sow thistle (Sonchus oleraceus L.) as a source of inulin-type fructans. The sequential ethanol and water extractions from their dry taproots were carried out. The amount of extracted fructans was defined by the resorcinol assay. The fructooligosacharides and inulin contents of the obtained extracts were analyzed by TLC and HPLC-RID methods. The total fructan content in the weed plant Sonchus oleraceus L. (19.6% dw) is higher than the fructan level in the roots of Lactuca serriola L (9.56% dw). In the ethanol extracts were observed the presence of monosaccharide glucose and fructose, high level of sucrose and trisaccharides 1-kestose. In the result of the carried analysis, we can conclude that the roots are rich source of fructans as the fructoligosacharides fraction dominates in ethanolic extracts. These plants could not only be consider as weeds, but it have to pay attention to their future possibility to be used as a potential source of fructooligosacharides with prebiotic effect in nutrition formula for animals and human.

Keywords: *fructoligosacharide, inulin, Lactuca serriola, Sonchus oleraceus.*

INTRODUCTION

Inulin is a polydisperse plant polysaccharide, member of fructan family, consisting mainly of β-(2→1) fructofuranosyl units (F_m), and a terminal α-glycopyranose unit (1→2) (GF_n) (Van Laere et al., 2002). The degree of polymerization (DP) of inulin varies from 2 to 70 (De Leenheer et al., 1994). Molecules with DP<10 are called oligofructoses or fructooligosaccharides (FOSs) (Figure 1) and they are a subgroup of inulin (Niness, 1999).

Inulin and FOSs are classified as soluble dietary fiber. They act as prebiotics, because stimulate growth of *Bifidobacteria.* Inulin is only hydrolyzed in small amounts in the stomach. In large intestine it is fermented by intestinal microflora into short-chain fatty acid (SCFA), lactic acid and gases (Gibson, 1995, Knudsen, 1995). Inulin-type prebiotics reduce blood levels of triglycerides (Roberfroid, 2005); prevent cardiovascular disease and osteoporosis (Delzenne, 2002). Inulin is helpful in the management of diabetes and blood sugar-related illness (Rumessen, 1998). In recent issues, inulin is presented as immunomodulator and anticancer agent (Barclay et al., 2010).

Depending on the conditions of extraction and the type of used raw material, a short-chain bioactive molecules (FOSs) or long-chain ones (inulin) could be achieved. Both they have different bioactivity as no digestible oligosaccharides of long chain length are typically less biodegradable than compounds of shorter chain length. Van Loo (2007) proposed that a combination of short-chain and long-chain fructans is physiologically more active that the individual fractions.

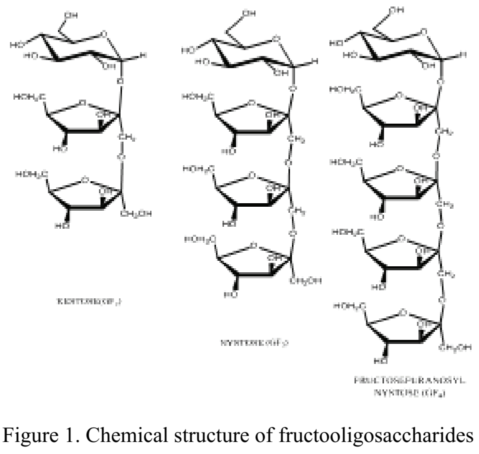

Figure 1. Chemical structure of fructooligosaccharides

Inulin serves as a reserve carbohydrate in underground part of the *Compositae* (*Asteraceae*) plants such as *Cichorium intybus, Inula helenium and Helianthus tuberosus* (Van Laere et al., 2002). Prickly lettuce (*Lactuca serriola*) and annual sow thistle *Sonchus oleraceus* L. also belong to this plant family.

Prickly lettuce (Lactuca serriola L.) is an annual or biennial plant, slightly foetid, that is commonly considered as a weed of orchards, roadsides and field crops. Many species in Lactuca are medical herbs, as well as wild vegetable. Scientists focused their research interest on searching for some promising compound with effectiveness and low toxicity for the benefit human`s health (Ren et al., 2004). The plant can be eaten as a salad, although it has a bitter taste. The young leaves can be eaten raw or cooked (Kleonikos, 2006)

Sonchus oleraceus L. is growing in cultivated fields and disturbed sites, ditch banks, bottomlands, city lots and alleys (Reaume, 2010). *Sonchus* wild food plants might be applicable in natural medicine and healthy food. *Sonchus oleraceus* L. and *Sonchus* sp.pl. eaten in several Italian regions, are cholagogue and laxative agents, due to their sesquiterpene lactones but also the high content of vitamin C, carotenoids and fatty acids of type ω-3 (Aliotta, 1981, Guil-Guerrero, 1998). In China, Sonchus wild vegetables are used mostly in infusion or decoction and are administered to treat acute icterohepatitis, cancer, inflammation, rheumatism, diarrhoea and snake venom poisoning (Dao et al., 2011). The underground roots of sow thistle store reserve carbohydrates, and inulin is the major storage carbohydrate in them (Lemna et al., 1990).

Figure 2. Photos of prickly lettuce (*Lactuca serriola* L.) and annual sow thistle (*Sonchus oleraceus* L.)

The variety *S. arvensis* can be used as a livestock feed and is considered to be highly nutritious for rabbits (Szcza-wenski et al., 1978). Boulos (1973) stated that *S. arvensis* roots can be used as a coffee substitute when is roasted.

According to Jana et al. (2010) the prebiotic effect of the *Taraxacum officinale, Sonchus oleraceus* and *Asparagus sprengeri* extracts on *L.lactis* and *L. reuteri* was higher than or equivalent to inulin - a commercial prebiotic, as *Sonchus oleraceus* exhibited the best prebiotic effect. It was the only plant to stimulate all the probiotics including *B. longum*.

In this context, the paper present an analysis of the fructooligosaccharides and inulin content in the roots of *Lactuca serriola* L. and Sonchus oleraceus L. from Plovdiv region of Bulgaria in order to study their inulin-type fructan content. This investigation aimed to present that these weeds can be potential and unstudied source of prebiotics.

MATERIALS AND METHODS

The roots of *Lactuca serriola* L. and Sonchus oleraceus L. were collected from Thracian valley near to Plovdiv (Bulgaria) during the months September and November in 2012 year. The underground parts were dried and ground into a fine powder.

All used reagents and solvents were of analytical grade scale. Carbohydrate glucose, fructose, sucrose, together with high purity 1-kestose and nystose, used as standards for the identification of low molecular weight oligomers have been purchased from Sigma-Aldrich (Steinheim, Germany). Fructooligosacchrides Frutafit®CLR, HD and inulin Frutafit®TEX were supplied by Sensus (Roosendaal, the Netherlands). Frutafit®CLR contains high level of oligofructoses with the average chain length of 7-9 monomers. Frutafit®HD - with the average chain length of 8-13 monomers. Frutafit®TEX was characterized with mean degree of polymerization DP 22. Inulin Raftiline®HP (DP~25) was purchased from Orafti (Belgium).

Moisture content of the dried ground roots was determined according to AOAC 945.32.

Dried roots of weed plants were extracted in a Soxhlet apparatus successively with hexane,

$CHCl_3$, and ethyl acetate to remove phenolic and lipophilic compounds (Olennikov et al., 2009). Then the residue of roots was dried and the extraction process was carried as follows: 0.45 g dry sample (roots) was put into a round bottom flask and was extracted three times with 95% (v/v) boiling ethanol. For the first and the second extraction, 40 ml 95% (v/v) ethanol were used and 20 ml for the third one. The duration of each extraction procedure was 60 minutes. The extracts were collected in 100 ml volumetric flask. The low-molecular carbohydrate fraction composed of fructose and FOSs was obtained in the ethanol extracts. For extraction of high-molecular fraction (inulin), the residue in the flask after ethanol extraction was extracted by three following extractions (40, 40, 20 ml) with boiling water as it was described above. The content of mono-, di-, oligosaccharides and inulin in the obtained extracts was analyzed by TLC in order to observe the extraction rate of fructans.

Thin-layer chromatography (TLC) of the obtained ethanol and water extracts from roots of prickly lettuce and annual sow thistle were performed on silica gel 60 F_{254} plates (Merck, Germany) with *n*-BuOH:*i*-Pro:H_2O:CH_3COOH (7:5:4:2) (v/v/v/v) used as a mobile phase. The spots were detected by dipping the plates into the solution with detecting reagent diphenyl-amine-aniline-H_3PO_4–acetone (1:1:5:50) (Lin-gyun et al., 2007) and heating at 120 °C for 5 min. As carbohydrate standards were used glucose, fructose, sucrose, 1-kestose, nystose, fructooligosaccharides (Frutafit CLR and HD) and inulin (Frutafit TEX and Raftiline HP) all of them in concentration 2 mg/ml. Thin-layer chromatograms were generated by densito-metry measurement of obtained spots with QuantiScan Version 3.0 software (Biosoft).

The fructan contents in ethanol and water extracts were analysed spectrophotometrically at wavelength 480 nm by resorcinol-thiourea reagent (Pencheva et al., 2012). The experi-ments were carried out on a Camspec M107 Vis spectrophotometer (UK).

The sugars and FOSs content in ethanol extracts was analyzed by HPLC. Chromato-graphic separations were performed on HPLC Shimadzu, coupled with LC-20AD pump, refractive index detector Shimadzu RID-10A, a column Supelcosil LC-NH2 (Supelco®, Sigma-Aldrich, Bellefonte, PA, USA) with pore size 5 µm and degasser Waters In-Line –IF (Milfrd, MA, USA). The separations were performed on an analytical aminopropyl silica column SUPELCOSIL LC-NH2 (250 x 4.6 mm i.d.) equipped with a guard column (2.5 x 4.6 mm i.d.) of the same filling. The mobile phase used for separation of glucose, fructose, sucrose and FOSs was acetonitrile/water (83/17 v/v). The column was placed into a temperature-controlled unit LCO 102 (ECOM spol. s.r.o., Czech Republic) maintained at 40 °C. All samples were filtered through a 0.45 µm filter. Injection volume of the sample was 20 µL and the flow rate of the eluent was 1.5 $ml.min^{-1}$with an isocratic mobile phase. Detection and identification of sugars and fructooligosaccha-rides were performed using RID detector that operated at 40 °C. The control of the system, data acquisition, and data analysis were under the control of the software program LC solution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan).

RESULTS AND DISCUSSIONS

The moisture content in the taproots of plants prickly lettuce was 8.46% and 10.41 % in the roots of annual sow thistle, respectively.

The results from determination of fructan content in the extracts from the underground parts of prickly lettuce and annual sow thistle were obtained by our developed ketose-specific spectrophotometric method with resorcinol reagent (Pencheva et al., 2012). On the base of our previous investigations of the extracts from dandelion, elecampane and topinambour, our observation during analysis have been shown high levels of low moleculecular fraction in ethanol extracts. Therefore, after ethanol pre-treatment of the samples in water extracts have been remained FOSs with longer chain length and inulin. The ethanol and water extracts obtained from *Sonchus oleraceus* L. (8.26 ±0.22 g/100 g dw and 11.30±0.09 g/100 g dw) contained big quantity of low molecular fraction than the same extracts obtained from *Lactuca serriola* L. The ratio between fructans in the ethanol and water extracts from roots of prickly lettuce is almost equal. Therefore, the low and high molecular fractions have been extracted at the same extent. In the result of our study we can conclude that from both plants *Sonchus oleraceus* is richer source of FOSs and inulin than prickly lettuce (Table 1 and Figure 3).

Table 1. Fructan content in the extracts obtained from the taproots of prickly lettuce and sow thistle (g/100 g dw1)

Plant type	Low molecular fraction (fructose, sucrose & FOS[1])	High molecular fraction (inulin)	Total fructants
		mean ± SD[3]	
prickly lettuce (*Lactuca serriola* L.)	5.39±0.22	4.17±0.50	9.6±0.86
annual sow thistle (*Sonchus oleraceus* L.)	8.26±0.22	11.30±0.09	19.56±0.14

[1]dw – dry weight; [2]FOS – fructooligosaccharides; [3]SD – standard deviation

The obtained results from TLC analysis of the ethanol and water extracts from the roots of prickly lettuce and annual sow thistle showed that extraction process in triplicate was efficient. Almost all carbohydrates presented in the samples have been successively extracted during these sequential extractions with ethanol and water used as solvents. All ethanol extracts (from 8 to 11 and from 16 to 19) contained fructose (R_f = 0.55), sucrose (R_f = 0.48) and FOSs which are equivalent to standards Frutafit CLR (7-9 oligomers) and HD (8-13 oligomers). The TLC analysis of the water extracts from the roots (12, 13, 14, 15, 20, 21, 22, 23) showed the presence not only of mentioned above FOSs, but also these extracts contained high molecular fraction of inulin with DP, similar to these of used as standards Frutafit TEX and Raftiline HP (DP 22-25). The water extracts obtained from the roots of annual sow thistle contained also and sucrose (R_f = 0.48) (Figure 3).

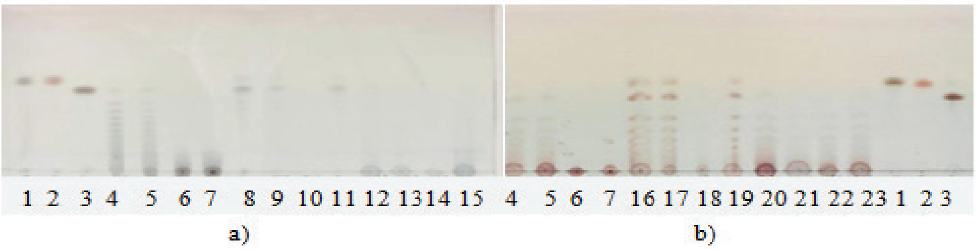

Figure 3. Thin-layer chromatography of fructans in 5 µl ethanol and 5 µl water extracts obtained from plants a) prickly lettuce (*Lactuca serriola* L.) and b) annual sow thistle (*Sonchus oleraceus* L.), standards 1-glucose, 2-fructose, 3-sucrose, 4 and 5-FOSs Frutafit CLR and HD, 6 and 7 - inulin Frutafit, TEX and Raftiline HP; 8, 9, 10, 11 – first, second, third and common ethanol extract from prickly lettuce; 12, 13, 14, 15 - first, second, third and common water extracts; 16,17,18 and 19 - first, second, third and common ethanol extracts; 20, 21, 22, 23 first, second, third and common water extracts from annual sow thistle.

The results obtained from densitometry analysis of the thin-layer chromatograms showed presence of high level of trisaccharides 1-kestose (R_f = 0.37) and tetrasaccharide nystose (R_f = 0.34) in ethanol and water extracts from the roots of prickly lettuce (*Lactuca serriola* L.) and annual sow thistle (*Sonchus oleraceus* L.). Except sugars fructose and sucrose, the extracts contained FOSs like commercial FOSs or inulin, used as standards. Solvent ethanol have been extracted FOSs until 9 monomer units (from GF3 to GF8). In the water extracts except FOSs with GF9 also dominate and high molecular inulin (Figure 4).

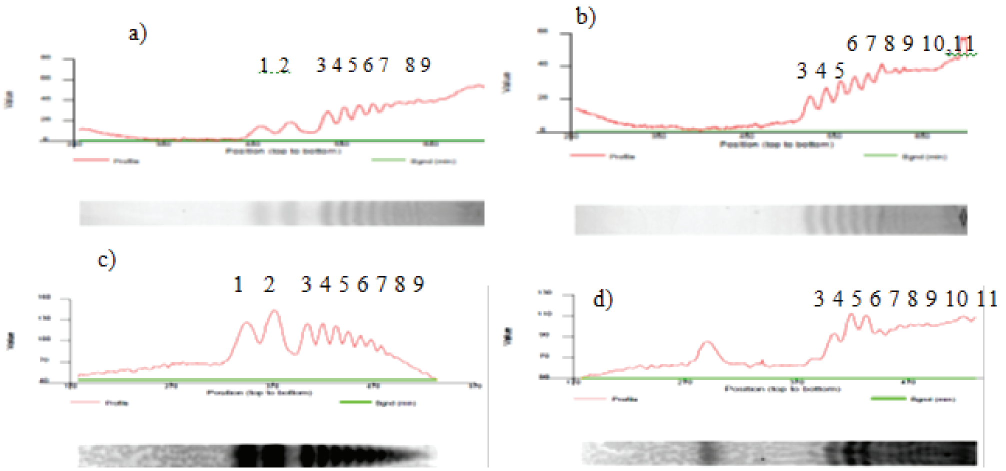

Figure 4. Thin-layer chromatograms of extracts from 5 µl a) ethanol and b) water extracts from roots of Sonchus oleraceus L. and 10 µl c) ethanol and d) water extracts from roots of Lactuca serriola L., where 1. fructose, 2. sucrose, 3.1-kestose (GF2), 4.nystose (GF3), 5.pentafructooligosaccharide (GF4), 6,7,8,9,10. fructooligosaccharides (respectively GF5, GF6, GF7, GF8, GF9) and 11. inulin

High-performance liquid chromatography with refractive index detection (HPLC-RID) has been widely used for determination of sugars and small oligosaccharides. After the ethanol extracts have been obtained from roots *Lactuca serriola* L. and *Sonchus oleraceus* L. these extracts have been analysed by the HPLC coupled with refractive index detector. These analyses help us to determinate the quantity of sugars and FOSs in their roots. The HPLC analysis proved the results obtained from the TLC analysis. The obtained chromatograms showed the presence of fructose (t_R=3,9 min), sucrose(t_R=6,1 min), 1-kestose (t_R=14,1 min) and nystose (t_R=20,9 min) in the ethanol extracts and also showed the presence of glucose (t_R=4,7 min) in them (The HPLC chromatogram of *Lactuca serriola* was not shown) (Figure 5).

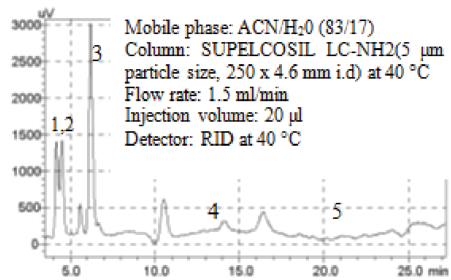

Figure 5. HPLC chromatograms of 95% (v/v) ethanol extracts of a) prickly lettuce (*Lactuca serriola* L.) and b) annual sow thistle (*Sonchus oleraceus* L.): 1.fructose, 2.glucose, 3.sucrose, 4.kestose and 5.nystose

The obtained results from HPLC analysis showed that the ethanol extract from roots of *Sonchus oleraceus* L. contained more 1-kestose and nystose (1.25 and 1.28 % dw, respectively) than prickly lettuce (Table 2).

Table 2. Mono- and oligosaccharides content (% d.w) in the ethanol extracts obtained from the roots of *Lactuca serriola* L. and *Sonchus oleraceus* L.

Plant	fructose	glucose	sucrose	1-kestose	nystose
prickly lettuce (*Lactuca serriola* L.)	1.78	0.91	2.23	0.80	0.65
sow thistle (*Sonchus oleraceus* L.)	2.03	1.31	3.92	1.25	1.28

Lactuca serriola L. and *Sonchus oleraceus* L. contains in their roots high amount of frucooligosaccharides. The results of our research showed that the underground parts of annual sow thistle is rich source of trisaccharide kestose, tetrasaccharide nystose, FOSs and inulin. All these inulin-type fructans possess well-pronounced prebiotic effect. These taproots could be used in feed and foods to increase the dietary fiber content in them.

Our research explained and proved the statement of Jana et al. (2010) that *Sonthus oleraceus* L. possess the best prebiotic effect and stimulate growth of *B. longum*.

CONCLUSIONS

The results from our analysis of the ethanol and water extracts obtained from the roots of *Lactuca serriola* L. and Sonchus oleraceus L. showed that these plants contain inulin-type fructan. Because of the absence of information in literature about the fructooligosaccharides and inulin contents in their underground parts for us it was a challenge to investigate these weed plants eaten as a salad in some countries in the world. The roots of annual sow thistle (Sonchus oleraceus L.) contains much more total inulin-type fructans (19.6 g/100g dw) than the roots of Lactuca serriola L. (9.56% dw) The levels of 1-kestose and nystose are higher in the ethanol extract of the annual sow thistle. The both plants are rich source of fructooligosaccharides that are in much more content in the ethanol extracts. The water extracts contain high molecular fructooligosaccharides and inulin. The findings of the current study showed that these two widespread weed plants are potential source of fructooligosaccharides (DP 3-5) and can be used as a new source of prebiotics that can find application in human or animal nutrition.

ACKNOWLEDGEMENTS

We would like to kindly acknowledge prof. Atanas Pavlov and PhD Ivan Ivanov for the access to HPLC chromatograph in their lab.

REFERENCES

Aliotta G., Pollio A., 1981. Vitamin A and C contents in some edible wild plants in Italy. Riv. Ital EPPOS, 63, 47-48

AOAC International, 2007. Official methods of analysis, 18th edn, 2005;. AOAC International, Gaithersburg, MD

Barclay T., Ginic-Markovic M., Cooper P., Petrovsky N., 2010. Inulin - a versatile polysaccharide with multiple pharmaceutical and food chemical uses. Journal Excipients and Food Chem., 1 (3), 27-50

Boulos L., 1973. Revision systématique du genre *Sonchus* L. IV. Sous-genre 1. *Son-chus*. Bot. Not. 126, 155-196.

Dao-Zong Xia , Xin-Fen Yu , Zhuo-Ying Zhu & Zhuang-Dan Zou, 2011. Antioxidant and antibacterial activity of six edible wild plants (Sonchus spp.) in China, Natural Product Research. 25:20, 1893-1901

De Leenheer L., Hoebregs H., 1994. Progress in the elucidation of the composition of chicory inulin, Starch-Starke. 46, 193-196

Delzenne N., Williams C., 2002. Prebiotics and lipid metabolism. Curr Opin Lipidol 13, 61–67

Gibson G., Roberfroid M., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition, 125, 1401–1412

Guil-Guerrero JL., Gimenez-Gimenez A., Rodriguez-Garcia I., TorjiaIsasa M., 1998. Nutritional composition of Sonchus species (*S. asper* L., *S. oleraceus* L. and S. tenerrimus L.). Journal Sci Food Agric, 76, 628-632.

Jana G. K. et al., 2010. Plant Derived Probiotics and Prebiotics: Possibilites are Immense. International Journal of Chem. and Anal. Science, 1(8), 177-180

Kleonikos G. Stavridakis, 2006. Wild edible plants of Crete - ΗΆγριαβρώσιμηχλωρίδατηςΚρήτης. Rethymnon Crete. ISBN 960-631-179-1

Knudsen, K., Hessov I.. (1995). Recovery of inulin from Jerusalem artichoke (*Helianthus tuberosus L.*) in the small intestine of man. British J. of Nutr, 74, 101-113

Lemna, W., Messersmith, C., 1990. The biology of Canadian weeds. 94. *Sonchus arvensis* L. Canadian Journal of Plant Science, 70, 509-532

Lingyun W., Jianhua W., Xiaodong Zh., Da I., Yalin Y., Chenggang C., Tianhua F., Fan Zh., 2007. Studies of the extraction technical conditions of inulin from Jerusalem artichoke tubers. J. of Food Engineer, 79, 1087-1093

Niness, K., 1999. Inulin and oligofructose: What are they? Journal of Nutrition, 129, 1402S-1406S

Olennikov D., Tankhaeva L., Rokhin A., 2009. glucofructans from *Taraxacum officinale* roots. Chemistry of Natural Compounds, 45 (2), 143.

Pencheva D., Petkova N., Denev P., 2012. Determination of inulin in dough products. Scientific works of UFT: "Food science, engineering and technologies", UFT Academic Publishing House, Plovdiv, Volume LIX (59), 339-344.

Ren Y., Zhou Y., Ye Y., 2004. Chemical components of *Lactuca* and their bioactivities. Acta Pharmaceutica Sinica, 39 (11), 954-960

Reaume Tom, 2010, Annual Sow-thistle *Sonchus oleraceus* Asteraceae—Aster family, Nature Manitoba

Roberfroid M., 2002. Global view on functional foods: European perspectives. British Journal of Nutrition, 88, Suppl. 2, 133 - 138.

Rumessen J., Gudmand-Høyer E., 1998. Fructans of chicory: intestinal transport and fermentation of different chain lengths and relation to fructose and sorbitol malabsorption. Am J Clin Nutr; 68, 357–64

Szczawenski, A.F. and Turner, N.J. 1978. Edible garden weeds of Canada. Nat. Mus. Nat. Sci. Ottawa, Ont, 184

Van Laere, A. and Van Den Ende, 2002. Inulin metabolism in dicots: chicory as a model system. Plant, Cell and Environment, 25, 803-81

Van Loo, J., 2004. The specificity of the interaction with intestinal bacterial fermentation by prebiotics determines their physiological efficacy. Nutr Res Rev, 17, 89-98